

The Hypothesis of Formative Stuff as Applied to *Bryophyllum Calycinum*

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HYPOTHESIS OF FORMATIVE STUFFS AS APPLIED TO BRYOPHYLLUM CALYCINUM¹

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(WITH TEN FIGURES)

Introduction

The obscure problems of organ differentiation in plants and animals have prompted considerable effort toward the characterization and identification of a specific substance, or specific substances, fundamentally responsible for the development of the different organs. Several investigators have tried to determine the rules governing the production and movement of these hypothetical substances, and to correlate their movements in the organism with organ development. In the literature of the subject these substances have been known by the very descriptive title of *formative stuffs* or *specific substances*.

It is obvious from the developmental history of any of the higher plants that the several distinctly different organs of each individual are all derived from cells of one kind, and, in the last analysis, from one cell. The problem is, does the individual possess different substances, each of which is responsible for one kind of organ of the plant? Is there a specific substance, or formative stuff, whose sole function is the formation of roots, another substance responsible

¹Contribution no. 189 from the Botanical Laboratory of the University of Michigan.

for the leaves, and still another responsible for the production of flowers? Or is there but one substance, or group of substances, whose varying concentration or environmental relationships determine differentiation? Assuming for the moment the simpler and cruder hypothesis of individual formative stuffs for the various organs, we must then provide a rational explanation for their localization in and movement about the plant. They must move from their place of origin to the points where they function as organ builders.

It is obvious that grave difficulties beset one who accepts what seems, at first sight, so simple and adequate a hypothesis. The nature of the difficulties can best be understood by a review of the experimental work upon which the hypothesis of formative stuffs has been based, and by a statement of the complications of the hypothesis which become necessary if all of the facts are to be met. It is found that a more modern point of view proves in the end to be more adequate and more comprehensible.

Much of the work on organ differentiation in plants in the past has been done with *Bryophyllum calycinum* Salisb., because of its peculiar production of new shoots from the notches of the leaves. Since this plant was very well adapted to a continuance of work where former investigators had left it, the writer chose it as the material for a large series of new experiments. The use of *Bryophyllum* had the further great advantage that it would enable the work of other investigators to be reviewed, a very necessary matter, since certain results reported in the past have seemed inconsistent with one another and not in accord with the general experience of plant propagators. The historical review which forms an introduction to the writer's own work will therefore deal most fully with the past investigations on *Bryophyllum*.

Morphology and physiology of *Bryophyllum*

Bryophyllum is a small genus of succulent plants belonging to the Crassulaceae. The species are natives of the tropics and cannot withstand the frosty weather of the northern states. The leaves are opposite, simple or pinnately compound. The members of this genus are peculiar because of the sprouting habit of their leaves.

If a leaf is removed from the plant, and laid in a moist, warm place, young plants will grow from the notches in the margin of the leaf. This new growth starts in about five to six days after the leaf is detached. This is the easiest method of propagating these plants, for, a few weeks after the new growth is well started, the little plants can be potted off. It frequently occurs that, in greenhouses



FIG. 1.—*B. calycinum*, showing growth from notches of leaf which has been immersed in water.

where these plants are raised, leaves break off from the plant and fall to the soil beneath the plant, and growth starts from the notches of the fallen leaves. Fig. 1 shows a *Bryophyllum* plant (*B. calycinum*), while in fig. 2 are shown several leaves and the new plant growth which has come from some of the notches of these leaves.

In addition to the meristem in the notches of the leaves, each node of the stem at the axil of the leaf is potentially capable of a new plant growth. There are not really buds at these places, but unusual formations of meristematic tissue. These formations of the stem are not apparent to the naked eye, for they are buried in the recesses where the petioles emerge from the stem. There is

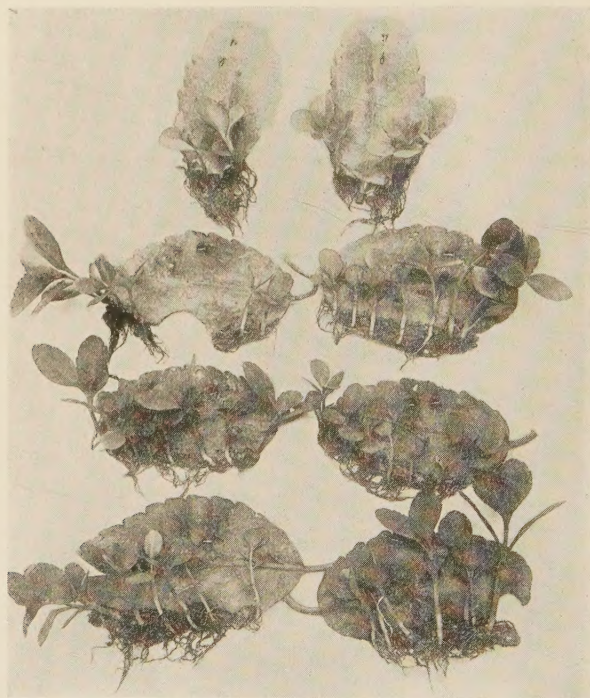


FIG. 2.—*B. calycinum* leaves, showing shoot growth from notches which were in contact with or beneath moist sand; also showing no growth from the other notches.

one on each side of the node. The location of this meristematic tissue in the leaf is easily determined, for it always occurs in the notches in the edge of the leaf, and this small sector in each notch is highly pigmented a dark brown.

These leaf groups of meristematic tissue can hardly be called buds, so that this form of propagation must be considered as growth occurring from meristematic tissue in unusual places; neither can

we use without qualification the term regeneration in describing this phenomenal growth, for it is clearly a case of vegetative reproduction and not the regeneration of a removed part. It is quite a different matter whether we remove a part of an organ and have that part replaced by a new structure of similar nature, or whether we have several new individuals arising from a part. The term regeneration has been freely and widely used in speaking of this form of propagation, and this fact will have to be kept in mind in the review of the history of the work.

Historical review

The hypothesis of formative stuffs to account for organ differentiation was originated by SACHS (9). He thought of formative stuffs as special substances potentially capable of determining the form which organs assume, and states the case as follows:

The substances for the formation of organs (apart from the germinal stages where they proceed from the reservoirs of reserve material) are produced in the foliage leaves by assimilation, and pass out thence into other parts of the plant. So long as a vigorous main bud of the shoot is present, mixtures of substances suited chiefly for the formation of shoots pass into it from leaves, whereas the substances fitted for the formation of roots flow in the opposite direction, into the roots which already exist.

SACHS speaks of flower forming stuffs, as well as root and leaf forming stuffs, and in general of formative stuffs for each organ of the plant. These are thought of as being capable of moving or being moved about the plant.

Authors subsequent to SACHS down to about 1902 do not add notably to his conception of formative stuffs, either accepting it without amplification or neglecting it. In 1902 GOEBEL (3) published an important work on regeneration in plants in which he refers to building stuffs which he considers food material. He accounts for the shoot and root development in the leaves of *Bryophyllum* as due to correlation between the growing parts at the apex of the stem and those in the notches of the leaves. The correlation is connected with the stream flow, in some cases with the water stream, in other cases with the food stream. He gives experimental evidence to support his view. GOEBEL's conception is essentially

the modern one, which differs from that of SACHS in that it squares with the modern physico-chemical theory.

GOEBEL also found that separation of the leaf of *Bryophyllum* from the mother plant is necessary before the growth of the leaf buds will take place. He quotes WAKKER, however, as having secured this bud growth from leaves which were not severed from the parent plant, simply by immersing the leaf in water. GOEBEL found that he himself was not able to secure the growth of the shoots from attached leaves when these leaves were kept in moist air, neither when they were overlaid with damp filter paper, nor when they were buried in damp earth. In all of his experiments it was necessary to interrupt the stream flow in some way to permit the development of the leaf meristem. By cutting the midrib of an attached leaf he interrupted the stream flow, and this leaf, although still attached, produced shoots when immersed in water.

In 1915 LOEB (5) published the first of a series of articles on the various phases of regeneration from *B. calycinum*. In this work he studied the phenomena of inhibition of regeneration in this plant. He found that the behavior was governed by the following rule:

If an organ *a* inhibits an organ *b*, organ *b* often accelerates and favors the regeneration of *a*. This rule is best understood on the assumption that the inhibiting organ receives something from the inhibited organ necessary for regeneration.

It is interesting to note that LOEB should choose a case of vegetative reproduction in the plant kingdom on which to formulate rules governing regeneration, since vegetative reproduction and regeneration are two entirely distinct processes, probably involving different factors. He conceives of a controlling influence which a whole individual exerts upon its parts. This accounts for the fact that a part may regenerate when cut from the whole organism, but not when it is still attached to the whole. The whole inhibits regeneration from the part while the two are intact. He finds that not only the whole organism but a small part of the whole, such as a piece of the stem, inhibits growth of any portion attached to this part. A piece of the stem of *Bryophyllum* may inhibit growth from the notches of the leaf attached to this stem piece. He further found that this was true even when the leaf was cut with lateral incisions,

thus showing that the flow of material from the leaf to the stem "must have taken place along a zigzag path in the leaf." In 1916 GOEBEL (4) published a critique of LOEB's paper, showing that some of his methods were faulty, and that some of his results had been published earlier by GOEBEL. Additional experiments were given by GOEBEL to show that, when cuttings were made from the stem of *B. calycinum*, the cuttings composed of a pair of leaves with intervening piece of stem, the piece of stem did not inhibit the formation of roots and shoots from the foliar notches.

In 1918 LOEB (6) published the results of further experiments in which he cut one leaf into as many pieces as the leaf had notches, and compared the mass growth from the notches of this leaf with the mass growth from the notches of another leaf which was not cut into pieces. Both leaves were kept on moist filter paper. The cut leaf gave new shoot growth from practically every notch, while the other leaf formed only four shoots. Concerning this, LOEB says:

We assume that in the latter leaf the shoots which grow out first inhibit the growth in the other notches. . . . Our contention is that this inhibition in leaf six [the uncut leaf] is due to the absorption of all of the material available for shoot formation by the four notches that happened to grow out first, thus depriving the other notches of the material needed for the growth of shoots.

In repeated experiments LOEB found that in the two cases, of slashed and unslashed detached leaves, the masses of shoot growth were practically the same weight. In the application of his rules regarding inhibition of regeneration, LOEB (5) makes the following statement:

These rules give us some basis on which we may try to form a preliminary idea of the nature of the mechanism of inhibition. As we mentioned already, the rules are comprehensible if we assume a flow of certain (possibly specific) substances (or formed cells) from the places where the dormant buds are already to grow, or the prevention of such a flow toward these dormant buds.

The migration of formed cells from one part of a plant to another is impossible, of course, since all plant cells are surrounded by a rigid wall, making migration, such as that of leucocytes in animal tissue, quite inconceivable.

Since the rules presented by LOEB do not allow for the possibility of leaves of *Bryophyllum* growing shoots from the notches while the leaf is still a part of the whole plant, it is interesting to note the short report of Miss BRAUN (1). She cites a case in which a leaf of *B. calycinum* on a normal potted plant produced shoots without immersing the leaf in water. LOEB (7) explains the growth in this case (quite unjustifiably) on the ground that the plant in question was sick and so had not normal circulation.

In 1919 a short article reporting preliminary work by CHILD and BELLAMY (2) was published. They assume the reality of an inhibiting power of the whole on a part; that the growing tip inhibits the growth of the dormant buds in the leaf. Their method of experimentation was to interfere with or interrupt this inhibiting factor in its course between the growing stem tip and the leaf. They found that a cold compress applied to the petiole effected the block or check of the inhibiting force. In this manner virtual isolation of the leaf, so far as the inhibiting force was concerned, was effected, and resulted in the growth of shoots from the leaf thus isolated when it was immersed in water. Their conclusions were as follows:

It appears at least highly probable that the inhibiting action of the growing tip, a leaf, or other active region of a plant, depends for its passage from point to point upon metabolically active protoplasm rather than upon purely physical transportation in the fluids flowing through preformed channels in the plant. In other words, the mechanism of this physiological correlation appears to possess at least certain of the characteristics of a transmissive or a conductive as distinguished from a purely transportative mechanism.

A close examination of the experimental work recorded on the subject reveals the fact that there is great need of a thorough study of *Bryophyllum* and the correlations involved in its process of vegetative reproduction. The laboratory findings of certain of the investigators are at variance with those of others. GOEBEL found that separation of the leaf from the mother plant was necessary before growth could be induced from the notches of the leaf, while WAKKER is credited with the converse experience. Again, LOEB's findings confirmed GOEBEL's, while in the still more recent work of CHILD and BELLAMY, shoot growth was secured from the

notches of the leaf while the leaf was still attached to the parent plant. Again, GOEBEL found that by making one incision through the midrib of an attached leaf, growth would occur from the notches of that leaf when it was immersed in water. On the contrary, LOEB found that a small piece of the stem inhibited growth from the leaf notches, even after he had made several incisions in the leaf. Simple as the experimentation concerned with this phase of the question may be, therein is involved the theory concerning the movement of formative stuffs through the conducting systems of the plant. These are but examples of several instances of variance in the experimental data at hand on the subject, hence the need of a comprehensive study of the subject. It is hoped that the following experiments, frequently repeated and substantiated, may form a basis for conclusive and constructive ideas on the subject.

An entire, normal plant of *B. calycinum* was used, the crenate leaves of which, both simple and compound, are known to be potentially capable of the growth of new plants from the marginal notches. It is generally known that the leaves are disconnected from the parent plant before this new growth occurs, so the question arises, is there a correlation between the severance of the leaf from the plant and the new growth? If so, what is this correlation?

Further experiments on *Bryophyllum calycinum*

EXPERIMENT I.—The first question which naturally arises is: Does the leaf in general possess the power of new plant growth, or is this power definitely located in certain parts of the leaf? It is not an assumption that leaves of *B. calycinum*, when removed from the plant and placed on a moist substratum in a warm place, will grow new shoots from several, if not all, of the notches of the leaf.

Twenty leaves were removed from plants and the petioles cut from the leaves. From each notch of each leaf a small sector of the leaf tissue was removed by means of a sharp razor. Caution was exercised in the removal of this tissue to insure the removal of the very smallest portion of the leaf necessary, and still withdraw all of the meristematic tissue at these points. The comparative size

of the sections of tissue removed may be seen in figs. 3 and 4, the latter showing uncut notches. These twenty leaves were divided into two sets of ten each. Those of one set were implanted on moist sand, those of the other set were suspended vertically in damp chambers with the petiolar third of each blade in water. Except for the growth of root from the base of the midrib, as shown



FIG. 3.—*B. calycinum* leaves from which meristematic units have been removed, showing growth of roots from base of midrib; also showing lack of foliar shoots.

in fig. 3, there was no growth from these leaves. The experiment was continued for several months, in which time several of the leaves rotted. The small sectors which had been removed from the periphery of the leaves were placed in a damp chamber, and the great majority started plantlets in a few days.

This experiment not only shows the definite location of the meristematic tissue in the leaf, but also indicates that the potentiality for growth of shoots from the leaf is localized in these small

sectors of meristematic leaf tissue which were removed. Further, that the factor responsible for growth is something formed and centralized when the leaf is on the plant, and that a subsequent or continuous formation of this factor does not occur, for, if it did so, growth would have occurred subsequent to the removal of the sectors of tissue.

EXPERIMENT II.—Since there are meristematic units in the notches of the leaves of *B. calycinum*, the questions arise, what factors control the growth, or the dormancy, of these units? Why is it that the units do not grow while the leaf of which they are a part is attached to the normal plant? Does the plant or any organ of the plant exert an inhibiting influence on these units of meristematic tissue? Are they dependent upon certain specific substances or formative stuffs for their growth, which the plant, or any organ of the plant, consumes at their expense? It has previously been seen that the opinion of some is that the

growth of these units is dependent upon certain specific substances or formative stuffs, and that in the normal plant this formative stuff is used by the other growing parts of the plant with the result that the meristematic units of the leaves remain dormant. It has further been stated by some that not only the other growing parts of the plant inhibit this growth from the leaf notches, but even parts of the plant, such as a piece of a stem attached to a leaf or the axillary buds of the piece of the stem, may inhibit. As the reason for this was not clear, such experiments as would throw some light on the subject were repeated many times.



FIG. 4.—Leaf of *B. calycinum*, showing growth of roots from base of petiole; leaf continuing to live normally, and no evidence of germination of meristematic units in notches.

Twenty leaves, each with a piece of the stem attached (fig. 5), and with axillary buds removed from each stem piece, were suspended in a damp chamber for the purpose of noting what, if any, inhibitory effect this stem piece would have over the growth of the meristematic units of the leaves. For each leaf of this culture, its normally opposite leaf without a stem piece attached was also suspended in the damp chamber for control observation. It was surprising to note with what uniformity growth occurred from all the leaves in the damp chamber. There was no noticeable difference



FIG. 5.—Leaves of *B. calycinum*, with stem pieces attached to petioles, with both axil meristems removed, showing growth of foliar shoots.

between the growth from the leaves with the stem piece attached and from the control leaves. Figs. 5 and 6 show ten of these leaves with the stem piece attached in each case and with each leaf showing shoot growth from the notches nearest the water. A similar experiment was tried with leaves having the stem pieces attached and having neither stem bud removed. Growth occurred from the opposite axillary bud, but evidently with no inhibiting effect upon the meristematic units of the leaves, for each leaf gave growth from the notches nearest the water as in the previous case, and as did the control leaves in this experiment. Fig. 7 shows five leaves of this experiment with the shoot growth occurring

simultaneously from the notches of the leaves and the opposite axillary buds. A third culture was set up, repeating the two



FIG. 6.—Leaves of *B. calycinum*, with stem pieces attached, with only opposite axil meristem removed, showing growth of foliar shoots.

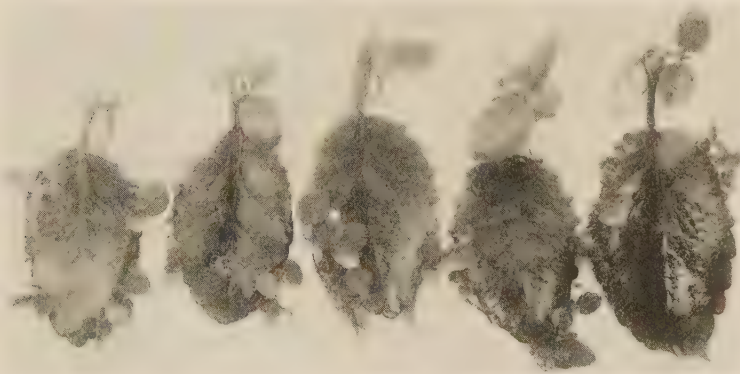


FIG. 7.—Leaves of *B. calycinum*, with stem pieces attached, with neither axil stem bud removed, showing growth from leaf notches as well as from opposite axil meristem.

foregoing experiments at the same time in the same damp chamber, and having a control leaf for each case. The growth in this culture confirmed the results of the two previous experiments.

All of these experiments, however, with their leaves suspended petiole upward in moist air above water, entail unnatural and unusual conditions. The leaf severed from the plant and placed horizontally on a moist substratum will, in the same length of time (about five to six days), show growth occurring from the notches. Also the axillary stem bud will grow under these more usual conditions. If the growth from the stem bud inhibits growth from the leaf it will surely do so under these conditions. Leaves with stem pieces and axillary buds intact were laid on moist sand, the leaf as well as the stem piece being in contact with the moist sand. In



FIG. 8.—Leaf of *B. calycinum*, showing that root growth occurs from meristematic units in notches of leaf, regardless of shoot growth from opposite axillary meristem.

every case the axillary stem bud grew, and in every case there was shoot growth from some of the notches of the leaf. Of course control leaves were used, being laid horizontally on the sand as the others were. In some cases there were a few more shoots on the control leaves than were on their respective leaves with stem pieces attached, but the converse was also true. There seemed to be no regularity about the shoot growth. Fig. 8 shows a leaf of this experiment.

The writer is thus unable to verify the work of LOEB, which indicates an inhibiting influence that the stem and the growth from the opposite axillary bud may have on the leaf shoots. LOEB has stated that it is the opposite axillary bud which inhibits the growth from the leaf. The writer knows of no morphological

reason why the opposite bud growth should inhibit and not the one at the base of the petiole of the leaf in question. Fig. 9 shows the growth from the axillary stem bud at the base of the petiole. It is very evident that this plant growing at the base of this petiole is fed from the leaf; there is no other source for food. This small plant, therefore, drawing its food from the leaf, should draw as strongly on the hypothetical formative stuffs of the leaf as any growth which may occur from a bud on the opposite side of an intervening stem, yet we see no signs of inhibition of growth from the leaf notches in fig. 9.



FIG. 9.—Leaf of *B. calycinum*, showing growth from meristem at base of petiole and from meristematic units of leaf.

EXPERIMENT III.—It has been asserted that growth at the apex of the stem of *Bryophyllum* inhibits growth from the leaf notches. LOEB explains the mechanism of this inhibition as a “sucking” of formative material away from the notches of the leaves by the growing parts at the apex, where the material is used for the development of new foliage. For the purpose of testing this hypothesis, the writer prepared five cultures of apical stem pieces bearing from one to three pairs of leaves. One specimen had one pair of mature leaves besides the small immature apical leaves, two others had two pairs, while the others had three pairs of the maturer leaves besides those which were immature. The five specimens were planted in

moist sand, with the base of the stem in each case inserted in the sand. As shown in fig. 10, each stem developed a vigorous growth of roots at the base. After the roots were well developed, sand was built up to the large leaves of each specimen, thus bringing the meristematic units of each leaf in contact with the moist substratum. If it were true that the growth of the new apical leaves and the apical bud caused inhibition of growth from the notches of the older leaves, we might surely expect such inhibition in these five



FIG. 10.—Growing tip of main stem of a *B. calycinum* plant, showing root growth from base of stem and shoot growth from notches of large leaves.

specimens, for in each case we have reduced the number of leaves this growing section might draw upon by cutting away, as we might say, the rest of the plant. Such was not the result, however, as may be seen in fig. 10. All specimens behaved in a manner similar to that shown in the figure. The specimen with one pair of large leaves gave shoot growth from 28 notches. One of the specimens with two pairs of large leaves gave shoot growth from 31 notches, while the other gave growth from 32 notches. One of the specimens having three pairs of large leaves gave shoot growth from 32 notches, while the other gave growth from 45

notches. It becomes evident, therefore, that at least in this case the growing apical parts of the plant did not inhibit growth from the notches of the leaves. In general we know that throughout the plant world we have dormant buds in plants, and that the awakening of these dormant buds often follows the removal of some growing part, but to say that growth in certain parts is the cause of dormancy in others may perhaps not be the correct analysis of the correlation.

EXPERIMENT IV.—Having segmented the plant into parts and having studied these parts, we are led to the conclusion that, at least in these parts of the plant, there is no one organ or group of organs inhibiting the growth of the meristematic units of the leaves under conditions under which moisture is supplied to the leaves externally. The question then is, how does the plant as a whole inhibit growth from the leaves? To answer this a set of cultures was prepared, using entire plants with one leaf of each immersed in a basin of water. Of course the immersed leaf in each case was not removed from the plant nor injured in any way. Fig. 1 shows a plant after the leaf had been immersed in water some days. In every case the leaf immersed in water gave shoot growth from some of its notches. This figure is a typical example of the experiment. GOEBEL (3) has reported that he was unsuccessful in obtaining shoot growth from the leaves in this manner, but he quotes WAKKER as securing such results. CHILD and BELLAMY (2) reported growth secured from the leaf while it was immersed in water and still attached to the plant. They explained this growth as due to the interference with the conducting or transmitting system by the cold application they used.

EXPERIMENT V.—A set of plants similar to those in experiment IV was placed in a dark room, which was ventilated with air forced in by a blower from the adjacent greenhouse. The plants were watered daily, but no water was placed in contact with any of the leaves. In other words, the plants were continued in their natural conditions except that they were deprived of light. In two weeks, growth of shoots was apparent from notches of many of the leaves. Combined with the data of experiment IV, these results point to the probable conclusion that the plant as a whole does not cause the dormancy in the notches of the leaves.

EXPERIMENT VI.—If the dormancy of the growing points in the notches of the leaves were dependent upon and a result of inhibiting factors from the growing parts of the plant, and we sever some of the leaves from the plant, we then remove this inhibiting force from these leaves and should obtain growth from some of the notches of the leaves, providing they are placed in a moist and light place.

After isolating several leaves from their stems, root growth was started from the base of the petiole of each by implanting the petioles in moist sand. The leaves were in an upright position and continued thus in a normal, healthy condition for six months without the occurrence of growth from any of the leaf notches. In this case it must be borne in mind that the lamina is not in direct contact with the moist sand, but the fact that the leaves continued apparently to perform their normal metabolic processes for six months (or indefinitely, for the experiment was not permitted to go longer), and at the same time showed no tendency toward producing new growth from the notches, is a result worthy of attention. In this case we have leaves similar in all respects to the leaves on the whole plant, except for the fact that they are no longer a part of the plant; they are no longer subjected to any inhibiting force exerted by the plant, and still the meristematic units remain dormant. We have severed the leaf, or the part, from the whole and still have dormancy. May we not therefore discard the whole (the plant in this case) as the source of the factor responsible for the dormancy of the meristematic units of the leaves? We have removed the plant from the leaf and still have dormancy; therefore, we have not removed the factor causing dormancy.

Continuing the experiment, at the end of six months the midrib of each leaf was cut through, the leaf being otherwise undisturbed. Three weeks later shoots had grown from some of the notches of each leaf; for example, 21 out of a possible 29 notches of one leaf produced shoot growth after the midrib was cut.

EXPERIMENT VII.—If we are to assume that shoot growth from *B. calycinum* leaves is dependent upon formative stuffs within the leaves, and that this formative stuff is transportable about the leaf, we may agree with LOEB that the growing of a certain number of

these units of meristematic tissue will withdraw from the leaf all of the formative stuff, and so deprive the remaining units of formative stuff necessary for their development. On the contrary, if good growth is secured from some of the notches of a leaf and subsequently good growth takes place from the remaining, or a portion of the remaining, notches of the same leaf, we cannot agree that his hypothesis applies, unless we assume that new formative stuff is continually being produced in the isolated leaf, and even in that case his "suction" hypothesis would hardly apply. By placing a portion of each leaf in contact with moist sand, good growth (plantlets 10 cm. high) was secured from those notches in contact with the moist sand, each leaf being severed from the parent plant. At the same time there was no growth from those notches which were not in contact with the moist sand. These new plantlets were then severed from the leaves, and the leaves inverted and again implanted in the sand, this time causing the heretofore dormant units to be in contact with the moist substratum. Within the usual time, eight days, there was a second crop of plantlets from these leaves, the growth again occurring from those notches in contact with the moist sand.

Another set of leaves was treated in a somewhat similar manner, but in this case, after those buds which were in contact with the sand had developed plantlets reaching 10 cm. or more, the leaves were not removed from the sand, but that part which extended free in air and the buds of which had remained dormant was brought in contact with the sand by slightly bending the leaves and building up the sand. The first plantlets to grow were not removed from these leaves. Again, within eight days, the heretofore dormant units of meristematic tissue unfolded into plantlets, as did the first to be favored with contact with the moist substratum. The first plantlets to grow did not deplete the leaf of that which was necessary for the growth of the other buds, nor did the growth of the units depend upon anything which had to be furnished from the leaf and which was subject to consumption or depletion by the first units to grow.

EXPERIMENT VIII.—Another series of isolated leaves was treated in the following manner. Some of the leaves were implanted with

only the very basal portion of the leaf blade beneath the moist sand. Others were almost entirely buried, and a grading series provided all possible intermediate conditions. Within ten days all of the notches of all of the leaves which were in contact with or beneath the sand gave shoot growth, while none of the notches above the sand did so. Fig. 2 shows a set of leaves treated in a similar manner, only some of the leaves were placed with their sides in the sand. Only those parts of the leaves which had been in contact with or beneath the sand showed growth. Here we have two unusual conditions in operation, one being the presence of water, the other being the absence of light.

EXPERIMENT IX.—To study the effects of the absence of light on the shoot growth from the notches of leaves of *B. calycinum*, a series of isolated leaves was treated in the following manner. One set of leaves was placed in a dry position before a south window; another set was placed in a dry dark box which was entirely light proof. Within ten days every notch of all the leaves in the light-proof box gave shoot growth, while only a very few of the notches of a few of the leaves in the light did so. As might be expected, the shoots which grew out in the dry box did not survive long. One may not assume, however, that the moisture of the air was the same in both sets of plants.

EXPERIMENT X.—It has been said by LOEB that approximately equal masses of new shoots are produced by equal masses of sister leaves, and from his work one may infer that "the material available for shoot formation" is of a specific nature. If such is the case, the mass of shoots affords a quantitative measure of the relative amounts of formative stuffs. If the mass of the leaf available for growth of shoots from the notches does not affect the number of notches giving initial growth, but on the contrary controls only the subsequent quantitative development of the plantlets, then the hypothetical "formative stuff" which is furnished the plant from the leaf simply fulfils the function of food stuff. If the number of notches to produce plantlets were dependent upon the mass of leaf tissue capable of being drawn upon by the meristematic units in the notches, one might conclude that the substance used in the initial formation of the shoot was derived from the leaf in general, and was specific, movable, formative stuff.

With this question in mind, a series of five sets of leaves was prepared, three similar leaves in each set, the total number of notches in each set being the same. One set was left entire. In the remaining four sets, a portion was cut from the central part of each leaf, varying from one-fifth of the leaf mass in one set to four-fifths in the last. All five sets were placed in glass damp chambers exposed to daylight. If growth from the notches of these leaves were dependent upon a limited amount of highly mobile formative material drawn by the meristematic units, or furnished to them, from the general mass of the leaf, the less the mass of the leaf the greater would be the inhibiting effect of the first buds to grow on the remaining ones of that leaf; the greater the mass, the greater the possibility of many buds starting. If, however, only the subsequent development of the plantlets, not their initial number, were decreased by the decrease of mass of leaf tissue, the question would appear to be one of food supply only.

Listing the sets numerically, beginning with that in which the leaves were left entire and ending with that set from which the greater portion of the middle of the leaf was removed, the number of notches giving initial growth was as follows:

- Set 1 gave initial growth from 38 notches, out of a possible 60
- Set 2 gave initial growth from 32 notches, out of a possible 60
- Set 3 gave initial growth from 44 notches, out of a possible 60
- Set 4 gave initial growth from 32 notches, out of a possible 60
- Set 5 gave initial growth from 35 notches, out of a possible 60

There appears to be no correlation whatsoever between the mass of the leaf and the number of meristematic units giving initial growth of plantlets, but in the further development of the plantlets it was very apparent that there was correlation between the mass of the leaf and the size of the plantlets. Those of the first set were larger than those of the second, those of the second larger than those of the third, etc.

Discussion

SACHS predicates different kinds of formative stuffs, a kind for each organ of the plant. These are capable of moving or being moved about the plant. GOEBEL speaks of building stuffs which he considers to be food material. He accounts for the shoot and root development in the leaves of *Bryophyllum* as due to correlation

between the growing parts at the apex of the stem and those in the notches of the leaves. The correlation is connected with the stream flow, the water stream in some cases, the food stream in other cases. In the application of his rules regarding inhibition of regeneration, LOEB assumes the inhibition to be due to "a flow of certain (possible specific) substances (or formed cells) from the places where the dormant buds are ready to grow, or the prevention of such a flow toward these dormant buds." The migration of formed cells from one part of a plant to another is impossible, of course, since all plant cells are surrounded by a rigid wall, making such migration as that of leucocytes in animal tissue quite inconceivable. These writers assume the existence of an inhibiting factor in the plant, functioning on the meristematic units of the leaves.

If we are to assume that shoot growth from *Bryophyllum* leaves is dependent upon formative stuff within the leaves, and that it is transportable about the leaf, we may agree with LOEB that the growing of a certain number of shoots from the leaf will withdraw from the leaf all of the formative stuff present, and so deprive the remaining growing points of formative stuff necessary for their development. This hypothesis allows for only one crop of shoots from a *Bryophyllum* leaf, for the first crop consumes all the substances required for the growth of shoots, automatically preventing any further shoot growth from the leaf. If a second or third crop of shoots is possible from the leaf, therefore, we cannot agree that his hypothesis applies.

As has been shown by experiment VII, a number of *Bryophyllum* leaves were so placed on moist sand as to permit growth from only half of the notches of each leaf. After the plantlets grown from these notches were well developed, one might have expected a depletion of formative stuffs within these leaves caused by the growing of these plantlets, but this was not found to be the case. A second crop of shoots was grown from the opposite side of these same leaves after removing the first. Again, as shown in the same experiment, a second crop can be grown equally well from a set of leaves without removing the first crop. Corresponding results may be obtained at any stage of growth of the foliar plantlets, from the initial stages of unfolding to the larger sizes several weeks old.

It appears evident, therefore, that growth from a portion of the meristematic units was not the cause of continued dormancy in the other growing points of the same leaf. It is further evident that the plantlets first to grow did not deplete the leaf of that which was necessary for the development of plantlets from the other notches.

It is frequently found that the plantlets which grow under like conditions from the same leaf show great variation in development, and some may even die, apparently due to other plantlets from the same leaf inhibiting their growth. Rather than attribute this inhibiting effect to the consumption of formative stuffs by some plantlets at the expense of others, however, is it not more probable that the nutrient supply only is used by those of more early growth at the expense of the later ones? That initial growth can be obtained from all the notches of the leaf is shown by experiment IX, but for a leaf to be able to support the subsequent development of plantlets from all its notches would appear to depend upon the availability of food.

LOEB has shown that approximately equal masses of new shoots are produced by equal masses of sister leaves. This is not a new discovery in plant physiology. It is well known that equal masses of plant tissue can be grown from equal amounts of endosperm and other food material. From LOEB's work, however, one may infer that the material available for shoot formation is of a specific nature. If such is the case, the mass of shoots affords a quantitative measure of the relative amounts of formative stuffs, but when, as shown in experiment X, we find that there is no correlation between the amount of leaf mass and the initial shoot growth, we are forced to the conclusion that the initial growth of the meristematic units of the leaves of *Bryophyllum* is not dependent upon specific substances derived from the leaf in general.

Passing from the isolated leaf as a unit, we may now consider briefly the leaf and a piece of the stem as a unit separated from the plant. With many repetitions of the various experiments with this unit, with and without the axillary buds, the writer was unable to verify LOEB's observations on similar experiments. As is shown by experiment II and figs. 5-7, 9, 10, there was growth from leaf

notches when water was present and available, regardless of growth from axillary buds or the presence of a piece of a stem.

Normal growth at the apex of the stem is quite a different thing from the growth of adventitious buds in parts of the plant, but, as shown in experiment III, even this growth at the apex does not inhibit the growth of plantlets from notches of the leaves when moisture is directly available to the meristematic units of the leaves. It may be possible that if the leaves have a limited amount of water to draw on, the active growing parts at the apex of the plant will draw so heavily upon this limited amount of water as to deprive the meristematic units of the leaves of their required amount for proliferation.

CHILD and BELLAMY start their work with the assumption that the growing tip does inhibit growth from other parts of the plant, and with this assumption they attempt to block this inhibiting factor in its course to a leaf of *Bryophyllum* by the use of a low temperature application to the petiole of the leaf in question, then by immersing the leaf in water the notches of the leaf give growth. In view of the fact that shoots will grow from leaves of *Bryophyllum* when immersed in water without the cold application, as is shown in fig. 1, can we be certain that their method of applying the cold application is responsible for the shoot growth they observed? Although some writers report that they are unable to secure shoot growth from leaves when immersed in water if intact with the plant, it is difficult to understand why this should be so; for it frequently occurs in greenhouses that such leaves, while in normal position on the plant, develop shoots without being immersed in water. Assuming again that the dormancy of the growing points in the notches of the leaves of *Bryophyllum* is dependent upon and a result of inhibiting factors from the growing apex of the plant, or from any part of the plant, or from the plant as a whole, it is obvious that these inhibiting factors are removed from influence in the leaf by severing the leaf from the plant. It is then to be expected that plantlets will grow from the notches of these leaves, since the factors which had caused their dormancy have been removed. In Experiment VI, however, it is shown that the meristematic units in the notches of the leaves of *B. calycinum* may be

kept in a dormant condition indefinitely without the influences of the inhibiting factors of the parent plant. It is found that leaves severed from the parent plant may continue in an apparently normal condition indefinitely, providing water supply is made possible by the growth of roots from the petiole of the leaf.

Let us return to the original assumption of some writers that the reason the meristematic units in the notches of the leaves remain dormant while the leaf is intact on the plant is that the plant inhibits the growth from the notches, because the growing part of the plant uses the formative material required for the growth from the notches of the leaf. We here remove the leaf from the plant, then remove the plant from the leaf, and still have continued dormancy. The cause of dormancy has been removed and still dormancy continues. In the face of this evident inconsistency, we are forced to the conclusion that we must allow for at least another cause of dormancy. Moreover, we have moved the possible seat of the factors for dormancy from the plant to the leaf.

The culture in question, that of an isolated leaf continuing an independent existence without the unfolding of the foliar shoots, is very interesting and of importance. Its great value lies in the fact that it eliminates many of the greatest difficulties of the problem. In place of LOEB's question, "What are the forces in the whole which exercise control over the part resulting in the prevention of regeneration?" we now ask, "What are the forces in the part which result in the dormancy of the meristematic units of this part?" The problem now involves the individual leaf, dormancy, and water. A new factor, namely, water supply, engages our attention instead of the hypothetical influence of the entire plant. Experiment VI shows that after six months of continued independent and apparently normal existence, the period of dormancy was broken by the cutting of the midrib, and hence, by removing the factor of water supply. Twenty-one out of a possible twenty-nine meristematic units grew after the midrib was cut, after the water was withheld from the conducting channels of the leaf. By cutting the midrib the water relations of the leaf were modified, and this probably set up a series of physical and chemical changes. To these changes, whatever they may be, we would ascribe the phenomena observed.

In his book on regeneration, MORGAN (8) makes the following statement:

There is another point which has been, I think, too much neglected, viz., that the production of food stuffs is itself an expression of changes taking place in the living tissues, and if the structure is changed so that it no longer produces the same substances it may then lead to the development of different kinds of organs. The difference in the regeneration of the apical and basal leaf of *Begonia* may be due to some difference in the structure of the protoplasm. The greater or smaller amount of starch produced in these leaves may be only a measure of, and not a factor in the result.

Attention is here focused on the protoplasm. Consider again the case previously mentioned, in which growth was obtained from a portion only of the notches of an isolated leaf, and later the remaining notches of the same leaf were induced to sprout by bringing them into contact with the moist sand. Again there is modification in the water relations of the leaf, and even parts of the leaf, and the probable physical and chemical changes in the protoplasm.

In experiment VIII a number of leaves provided a grading series so implanted in moist sand as to provide leaves with but a few notches beneath the moist sand, leaves nearly entirely buried, and intermediate conditions. In all of the leaves only those notches which were in contact with or beneath the sand gave rise to shoot growth. Fig. 2 shows a set of leaves of this culture. The buried portion only shows growth. There are two factors of possible influence in this case, (1) the presence of water, and (2) the absence of light. Both are capable of bringing about physical or chemical changes within the leaf.

1. *Presence of water.*—Several experiments have been cited wherein the presence of water appeared responsible for the growth of the dormant buds in the leaf of *B. calycinum*. Fig. 1 shows a culture after one leaf in a normal position on the plant had been immersed in water. The culture was grown in the greenhouse. Numerous cases have been cited by other investigators in which water was a factor in the development of growth from the leaf notches. It is evident that the presence or availability of water is of prime importance in this problem. It has been shown that water is perhaps the deciding factor in the case. One other is to be considered, however, the absence of light, and it may even be shown

that both water and light are only of secondary importance, influencing indirectly the resultant growth of the meristematic units of the leaves.

2. *Absence of light*.—To test the effect of light, or the absence of light, two sets of leaves were prepared. One set was placed in a dry location before a south window, the other in a dry lightproof box. As shown by experiment IX, the absence of light from the leaves, although in a dry place, resulted in so extensive a germination of the meristematic units that shoots sprouted from every notch of every leaf in the culture. In the same length of time there were but very few shoots started from those leaves before the window in the light, these also being without water. In experiment V is mentioned the fact that leaves still attached to the plants in the dark room produced shoots from some notches. The production of shoots in this case was by no means as extensive as that from the leaves in the lightproof box. This difference might be expected when we realize that physical or chemical changes would be less complete and slower in the leaves enjoying the transpiration and connection with the plant than in isolated leaves deprived of water. It appears, therefore, that, although the presence of water seems to be of great importance in this problem, the absence of light may be of equal importance. The single detached leaf with roots in moist sand is furnished water and nutrient, as is the leaf of the normal plant. In such a leaf, one should not expect physical or chemical changes other than those occurring in a leaf on a normal plant, but by cutting the midrib of the leaf having its own connection with the moist substratum we cut off the water supply and should expect these changes to occur in the leaf. Throughout all of the experiments reported, wherein foliar shoot growth has been recorded, this growth has followed some condition in which the leaf was subjected to conditions which would result in the metabolic changes mentioned.

Owing to the fact that most of the experimental work on this problem has been done with the use of water, the rôle of water becomes, perhaps, unduly magnified. We must not overlook the fact that the most abundant case of initial shoot growth from leaves occurred in the dark, in the lightproof box. In this culture are

provided the conditions which would bring about the most rapid and complete changes within the leaf, darkness and dearth of water. Use but one of these conditions alone and the changes will be slower and less complete. For example, the leaves lacking water, but in the light, would naturally continue normal metabolism longer than if the light had been shut off also. The great importance of the absence of light is lost sight of, because cultures of plant tissue do not lend themselves readily to dark room conditions. The starved plant in the dark is under very different metabolic conditions from the plant in the light, and this very fact emphasizes the importance of the effect of darkness.

There is one phase of this problem which should not be overlooked. It has been reported and frequently noted that leaves of apparently normal *Bryophyllum* plants will grow shoots from their notches while the leaf is still attached to the plant. Since such a case, reported by Miss BRAUN (1), conflicted with LOEB's "suction" hypothesis, he explained the growth on this particular plant as being due to the fact that the plant was sick and so had not normal circulation. It is a question whether such a plant, although it may be sick, is less healthy than portions of plants which have been cut off for experimental purposes. But grant that the plant is sick. What is the significance of that? Merely that the metabolic condition of the plant had changed, and if our inferences have been correct, plants such as this, sick, or undergoing metabolic changes, may be expected to show growth of shoots from the notches of the leaves. In cases of this kind the appearances of shoots on the leaves is not necessarily an indication that the conducting tracts of the plant are interrupted, but it is, perhaps, only an expression of the metabolic condition of the organ.

In formulating a conclusion, let us visualize the growing meristem in the notch of the leaf of *B. calycinum*. It is a small unit of meristematic tissue, formed in the notch of the growing leaf, surrounded by the normal metabolic processes of the growing leaf, and as long as the leaf continues to function normally and there is no change in the metabolic processes of the leaf, the meristematic unit undergoes no change. Interrupt the metabolic conditions of the plant, or the leaf, as has been done in many ways in the pre-

ceding experiments, and the meristematic units unfold and give rise to plantlets. External changes may bring about physical or chemical changes within the leaf. Water, absence of light, or even cold compress on the petiole may initiate a concomitant series of physical or chemical changes within the leaf. It is to these changes, whatever they may be, that we would ascribe the proliferation of the meristematic units.

In this analysis of *B. calycinum* it is found that the growing points in the notches of the leaves are small independent units, independent of other organs of the plant, and dependent only upon their imbedding media, as we might say. The indications are that there is no formative stuff or specific substance withheld from these growing points or fed to them.

Summary

1. The growth of foliar shoots in *B. calycinum* is possible only from the preformed buds in the notches of the leaf.
2. When these *Bryophyllum* plants are growing under normal conditions the buds in the leaves lie dormant.
3. There are no indications that this dormancy is due to the fact that these units are deprived of formative stuffs or specific substances, the availability of which would cause their germination.
4. The plant or any organ of the plant does not exert an inhibiting influence over these meristematic units.
5. The dormancy or the germination of these units is an expression of the metabolic condition of the organ of which they are a part.
6. The germination of these units is probably due to physical and chemical changes within the organ of which they are a part. Any factor or group of factors working together which set up this series of changes are indirectly responsible for the growth of the foliar plantlets or those from the axillary buds.
7. One of the conditions which starts the growth from the meristematic units of the leaf is very moist air or water in contact with the leaf.
8. The absence of light also brings about a condition which starts this growth.

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LITERATURE CITED

1. BRAUN, MISS E. L., Regeneration of *Bryophyllum calycinum*. BOT. GAZ. 45:191-193. 1918.
2. CHILD, C. M., and BELLAMY, A. W., Physiological isolation by low temperature in *Bryophyllum* and other plants. Science 50:362-365. 1919.
3. GOEBEL, K., Über Regeneration im Pflanzenreich. Biol. Centralbl. 22: 385-397. 1902.
4. ———, Zur JACQUES LOEB's Untersuchungen über Regeneration bei *Bryophyllum*. Biol. Centralbl. 36: 193-206. 1916.
5. LOEB, J., Rules and mechanism of inhibition and correlation in the regeneration of *Bryophyllum calycinum*. BOT. GAZ. 60:249-276. 1915.
6. ———, Production of equal masses of shoots by equal masses of sister leaves in *Bryophyllum calycinum*. BOT. GAZ. 65:150-174. 1918.
7. ———, Healthy and sick specimens of *Bryophyllum calycinum*. BOT. GAZ. 44:69. 1918.
8. MORGAN, T. H., Regeneration. p. 80. 1901.
9. SACHS, J., Lectures on the physiology of plants. Eng. ed., p. 521. 1887.

